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ORIGINAL ARTICLE

Cannabidiol presents an inverted U-shaped dose-response curve in a simulated public speaking test

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Objective: Cannabidiol (CBD), one of the non-psychotomimetic compounds of *Cannabis sativa,* causes anxiolytic-like effects in animals, with typical bell-shaped dose-response curves. No study, however, has investigated whether increasing doses of this drug would also cause similar curves in humans. The objective of this study was to compare the acute effects of different doses of CBD and placebo in healthy volunteers performing a simulated public speaking test (SPST), a well-tested anxiety-inducing method.
Method: A total of 57 healthy male subjects were allocated to receive oral CBD at doses of 150 mg (n=15), 300 mg (n=15), 600 mg (n=12) or placebo (n=15) in a double-blind procedure. During the SPST, subjective ratings on the Visual Analogue Mood Scale (VAMS) and physiological measures (systolic and diastolic blood pressure, heart rate) were obtained at six different time points.
Results: Compared to placebo, pretreatment with 300 mg of CBD significantly reduced anxiety during the speech. No significant differences in VAMS scores were observed between groups receiving CBD 150 mg, 600 mg and placebo.
Conclusion: Our findings confirm the anxiolytic-like properties of CBD and are consonant with results of animal studies describing bell-shaped dose-response curves. Optimal therapeutic doses of CBD should be rigorously determined so that research findings can be adequately translated into clinical practice.

Keywords: Anxiety; cannabis; cannabidiol; CBD; simulated public speaking

Introduction

Historically, *Cannabis sativa* has been used by many cultures for medical purposes. The plant contains more than 80 cannabinoids, of which two have been the most studied: tetrahydrocannabinol (THC), responsible for the main psychoactive effects of cannabis, and cannabidiol (CBD), which may account for up to 40% of the plant.[1,2]

CBD has been shown to be a safe compound with a favorable adverse effects profile.[3] A number of studies have shown that this cannabinoid has a broad range of therapeutic properties, including antipsychotic,[4] sleep-regulating,[5] antidepressant and mood-stabilizing,[6] antiepileptic,[7] anti-inflammatory[8] and analgesic[9] effects, in addition to improving Parkinson symptoms.[10] Reduced anxiety has also been one of the compound's most consistently observed effects in both animal models and human studies.[11-13]

Regarding the former, anxiolytic-like effects have been observed in different experimental models, including the Vogel conflict test,[14] contextual fear conditioning,[15] and the elevated plus maze (EPM).[16] Using the EPM, Guimarães et al. observed that CBD increases open arm exploration (an anxiolytic-like effect) at doses of 2.5 to 10 mg/kg).[12] Higher, doses, however, were ineffective. Inverted U-shaped dose-response curves in the EPM have also been described in mice[17] and in rats after direct injection of CBD into periaqueductal gray matter.[18] This dose-response curve pattern, which was also observed in other animal models such as an anxiety test in adult zebrafish[19] and forced swimming tests,[6] is similar to the known clinical effect of CBD. Despite these data, there are also contradictory results, Silveira Filho & Tufik[19] found no significant effects of high doses of CBD (100 mg/kg) in rats submitted to the Geller-Seifter conflict test. Another study registered anxiogenic-like effects

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from chronic CBD administration in rats (10 mg/kg) subjected to two behavioral tests: locomotor activity and conditioned emotional response.[20]

The presence of anxiolytic properties has been supported by some human studies. The pioneer study was conducted in 1982 and concluded that oral administration of CBD (1 mg/kg) to healthy volunteers attenuates the anxiogenic effect of THC (0.5 mg/kg).[21] In the 1990s, another trial compared the effects of ipsapirone (5 mg) and CBD (300 mg) to those of diazepam (10 mg) and placebo in healthy volunteers during a simulated public speaking test (SPST), an anxiety-inducing task. The results indicated that CBD attenuates speech-induced anxiety compared to placebo.[13] However, for 600 mg CBD, Bhattycharyya et al.[22] found only trend-level effects on anxiety as measured by the State-Trait Anxiety Inventory and no effect on a fearful faces task.

In a recent trial involving a SPST, our group found that pretreatment with CBD (600 mg) significantly reduced anxiety, cognitive impairment, and distress during speeches by patients with generalized social anxiety disorder, and significantly decreased alertness in the anticipatory period before the speech.[23] Corroborating these findings, studies using functional neuroimaging techniques showed that the anxiolytic properties of CBD are associated with the modulation of different limbic and paralimbic areas in both healthy volunteers[24] and social anxiety disorder patients.[25]

Taken together, the results obtained after single-dose administration of CBD indicate that this cannabinoid could be anxiolytic in humans, although no study has yet investigated whether increasing doses of this drug would also cause bell-shaped dose-response curves in humans. This lack of data is of particular importance for determining the effective therapeutic windows of CBD for treating anxiety and other clinical conditions. Therefore, the present study aimed to compare the acute effects of three different doses CBD vs. placebo in healthy volunteers during a SPST.

Methods

Subjects

A total of 57 healthy male subjects were allocated to receive different doses of CBD (150 mg; 300 mg; 600 mg) or placebo in a double-blind, randomized design. Only men were included in the present investigation to decrease possible gender-related variations. The groups were matched according to age, years of education, and socioeconomic status. None of the participants suffered from psychiatric disorders according to the Portuguese version[26] of the Structured Clinical Interview for the DSM-IV, clinical version (SCID-CV).[27] Individuals with a history of head trauma, neurological illness, or major medical illnesses (assessed with a semi-standardized medical questionnaire and physical examination) were not included. All participants were non-smokers and had not taken any medications for at least three months prior to study. None of the subjects had used marijuana more than five times in their lives (no use in the past year) and none had ever used any other illegal drugs. All subjects gave written consent to participate after being fully informed about the research procedures, which had been approved by the local ethics committee (process no. 0290/2012).

Experimental anxiety procedure

The SPST was performed according to the procedures described by McNair et al.,[28] with minor modifications introduced by Guimarães et al.[29] and Bergamaschi et al.[23] (Table 1). In brief, after arriving at the Laboratory of Psychopharmacology at the Faculdade de Medicina de Ribeirão Preto university hospital, the first part of the consent form was carefully read and, after a 15-minute adaptation period, baseline measurements (B) were taken, followed by a single dose of oral CBD (150 mg, n=15; 300 mg, n=15; 600 mg, n=12) or placebo (n=15) in a double-blind procedure. CBD or placebo

Table 1 Phases and procedures of the experimental session

Time	Phase	Procedure
0	Drug/placebo administration	VAMS, HR, BP, CBD (150 mg, 300 mg or 600 mg) or placebo
90	Pre-stress (PS)	VAMS, HR, BP
+ 0:10	Video instructions	The participant prepared a speech about public transportation and completed the 2nd part of the consent form
+ 0:12	Speech preparation	
+ 0:14	Anticipatory anxiety (A)	VAMS, HR, BP
+ 0:25	Beginning of speech	The participant gave his speech in front of a camera while viewing his image on TV
+ 0:27	Performance anxiety (S)	VAMS, HR, BP
+ 0:33	Speech continues	
+ 0:35	End of speech (F1)	VAMS, HR, BP
+ 1:05	Post-stress (F2)	VAMS, HR, BP

BP = blood pressure; CBD = cannabidiol; HR = heart rate; VAMS = Visual Analogue Mood Scale.

was administered one hour and thirty minutes before the test.

Pretest measurements (P) were made 90 minutes after taking the drug or placebo. Immediately afterwards, the participants received the pre-recorded video instructions for the test. The participants were told they had two minutes to prepare a four-minute speech about "the public transportation system of your city." They were also informed that the speech would be recorded on video and later analyzed by trained psychologists. Measurements of anticipatory anxiety (A) were taken just prior to the speech. Each participant then began speaking in front of the camera while viewing his image on TV (LED 3D 49' LG® 49LF6450 Full HD). The speech was interrupted in the middle and VAMS and BP measurements (S) were taken again. The remaining two minutes of speech were then recorded, with post-test measurements taken immediately after the end of the speech and 30 minutes later (F1 and F2, respectively).

CBD

CBD (150 mg, 300 mg, or 600 mg) in powder form (99.9% purity, kindly supplied by STI-Pharm, Brentwood, UK), was dissolved in corn oil.[3,5] The same amount of corn oil was used as placebo, and both the drug and placebo were packed inside identical gelatin capsules. These doses were chosen based on previous evidence that acute anxiolytic effects have been observed in healthy subjects given doses ranging from 300 to 600 mg of CBD.[24] The interval between the administration of the capsules and the measurements was chosen based on previous evidence that the plasma peak of an oral dose of CBD usually occurs 1-2 h after ingestion.[11,24,25,30,31]

Psychological measurements

The Portuguese version[32] of the Visual Analogue Mood Scale (VAMS),[33] was used to evaluate the state anxiety level and other subjective states during the test. It is a self-administered instrument in which subjects are asked to mark a point that identifies his/her current subjective state on a 100-mm straight line between two words that describe opposite mood states (e.g., calm-excited). The VAMS contains 16 items that can be grouped into the following four factors for factorial analysis: 1) anxiety (items calm-excited, relaxed-tense, and tranquil-troubled); 2) sedation (items alert-drowsy and attentive-dreamy); 3) cognitive impairment (items quick witted-mentally slow, proficient-incompetent, energetic-lethargic, clear headed-muzzy, gregarious-withdrawn, well-coordinated-clumsy, and strong-feeble); and 4) distress (items interested-bored, happy-sad, contented-discontented, and amicable-antagonistic).[23,34]

Physiological measurements

Systolic and diastolic blood pressure were measured with a mercury sphygmomanometer (Becton Dickinson, Brazil).

Data analysis

Clinical and demographic data were analyzed with Student's *t*-test for continuous data.

VAMS scores and systolic and diastolic measurements were transformed by calculating the difference between the score in each phase of the procedure and the pre-test measures for each participant (delta scores). Delta scores were then analyzed using repeated-measures analysis of variance (ANOVA) with group, phase, and group-phase interaction as factors. When sphericity was not attained, the degrees of freedom of repeated factors were corrected using Huynh-Feldt epsilon. Whenever a significant phase by group interaction occurred, comparisons among the groups were made at each phase using one-factor ANOVA followed by multiple comparisons with Bonferroni's test.

All analyses were performed in SPSS version 20.0 and with statistical significance set at $p < 0.05$.

Results

The demographic characteristics of the study participants are shown in Table 2. No significant differences were observed between the four groups.

Visual Analogue Mood Scale (VAMS): anxiety

Figure 1 presents variations in the VAMS anxiety factor for groups receiving placebo and different doses of CBD during the SPST. The repeated-measures ANOVA showed phase ($F_{2.48;\ 131.6} = 13.36$; $p < 0.001$) and group effects ($F_{3;53} = 2.714$; $p = 0.05$), but no significant interaction between group and phase ($F_{7.45;131.6} = 1.72$; $p = 0.1$). Comparisons between groups for each phase with post hoc tests showed lower anxiety levels in the group treated with CBD 300 mg in phase S than placebo ($p = 0.042$).

Table 2 Demographic characteristics of the groups

Variable	Placebo	CBD 150 mg	CBD 300 mg	CBD 600 mg	p-value
Group size (n)	15	15	15	12	
Age	24.5 (4.04)	24.2 (3.08)	24.6 (2.93)	22.6 (3.4)	0.95
BMI	26.4 (2.4)	25.4 (4.6)	26.26 (4.1)	25.3 (3.7)	0.70
Socioeconomic level, median (range)*	2.5 (1.0-4.0)	2.7 (1.0-3.0)	2.8 (1.0-3.0)	2.6 (1.0-3.0)	0.87
Years of education	14.3 (3.1)	15.2 (2.9)	14.8 (3.7)	15.3 (3.8)	0.83

Data presented as mean (standard deviation), unless otherwise specified.
BMI = body mass index; CBD = cannabidiol; SD = standard deviation.
* Socioeconomic level was assessed according to the Brazilian Socioeconomic Classification Criteria.[35]

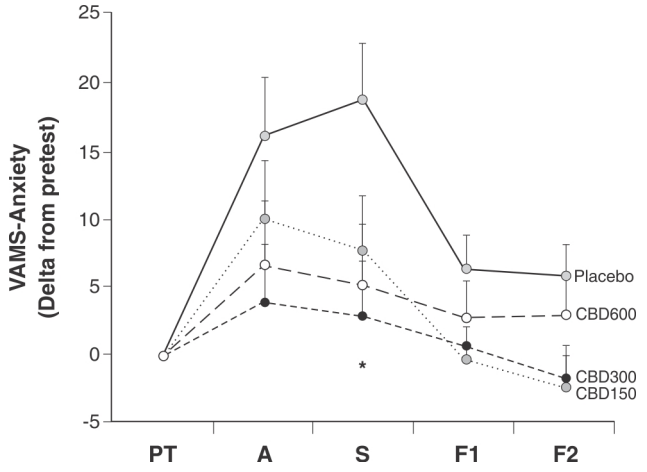

Figure 1 Visual Analogue Mood Scale (VAMS) anxiety factor scores in each phase of the simulated public speaking test (SPST) for groups treated with cannabidiol (CBD) 150, 300, and 600 mg or placebo (points in the curve refer to mean scores and vertical lines refer to mean standard errors).* Lower anxiety levels in the group treated with CBD 300 mg relative to the placebo phase ($p = 0.042$). PT = pre-test; A = anticipatory anxiety; S = speech; F1= post-test 1; F2 = post-test 2.

No significant phase, group, or group-phase interaction effects were found for the VAMS factors sedation, cognitive impairment, or distress. Neither the task nor the drug affected any other VAMS dimensions.

Physiological measures: BP

Regarding systolic BP, we found a significant phase ($F_{3.9;\ 211.314;\ 212} = 6.7$; $p < 0.05$) but not group ($F_{3;\ 53} = 0.7$; $p > 0.05$) or group-phase interaction effect ($F_{12;\ 211} = 0.99$; $p > 0.05$). Repeated-measures ANOVA showed significant phase ($F_{4;\ 212} = 2.8$; $p = 0{,}027$), but not group ($F_{3;\ 53} = 0.67$; $p > 0.05$) or group-phase interaction effects ($F_{12;\ 212} = 0.46$; $p > 0.05$) for diastolic BP.

Discussion

Confirming several preclinical and clinical studies, our results indicate that acute doses of CBD can decrease anxiety. This effect has also been previously demonstrated in studies using a SPST to induce anxiety in healthy subjects[13] and patients with social anxiety.[23] In the latter study, CBD (600 mg) treatment was associated with significant reductions in anxiety, cognitive impairment, and distress during the speech, as well as with reduced arousal during the anticipatory phase.[23]

Previous clinical investigations of the anxiolytic properties of CBD, however, have been based on single doses. In the present study, we tested increasing doses of this drug in a SPST. The VAMS results showed that CDB at 150, 300 and 600 mg produced an inverted U-shaped dose-response curve. In other words, the lowest or highest doses of CBD had little or no effect on SPST- induced anxiety and a significant response was only achieved with an intermediate dose. This dose-response pattern is consistent with evidence from CBD animal studies[12,16,18] and with our recent findings in a group of subjects who underwent a real-life public speaking test.[34]

The molecular and neural mechanisms involved in the anxiolytic effects of CBD are incompletely understood. One complicated topic is the bell-shaped dose-response curve produced by CBD in distinct biological systems. CBD has several pharmacological targets (for a review, see Campos et al.[36]), among which is its activation of TRPV1 receptors at higher doses. These receptors increase glutamate release, which could oppose 5HT1A- or CB1-mediated anxiolytic/antidepressant effects.[36,37] Campos et al.[37] showed that pretreatment with the TRPV1 receptor antagonist capsazepine turned the higher, ineffective dose of CBD into an anxiolytic one. This result suggests that activation of TRPV1 receptors at high CBD concentrations could be one mechanism involved in its anxiolytic bell-shaped dose response curves.

It is worth mentioning, however, that CBD bell-shaped dose response curves are not exclusively associated with the drug's anxiolytic effect. They have been described, for example, in depression using the forced swimming test[6] and the tail suspension test,[38] in compulsive behavior measured with the marble-burying test,[39] in schizophrenia using prepulse inhibition[40] and pain,[41] and in models of type 1 diabetes mellitus, inflammatory bowel disease, rheumatoid arthritis, and multiple sclerosis.[8] It is probable that different mechanisms are responsible for these effects.[37] In addition, these results indicate that the optimal dose of CBD could depend on the condition, which highlights the need to test different doses when planning human and animal studies.

Neuroimaging investigations in humans indicate that the action of CBD occurs in limbic and paralimbic brain areas, which are known to be associated with anxiety. Previous studies using single-photon emission computed tomography and functional magnetic resonance imaging have shown that the anxiolytic effects of CBD are associated with activity modulation in the amygdala, hippocampus, parahippocampal gyrus and cingulate cortex.[24,25,27] In addition, studies using animal models of anxiety suggest that facilitation of serotonin 5HT1A receptor-mediated neurotransmission in defense-related areas[6,17,37,42] could be a major mechanism responsible for the acute anxiolytic effects of CBD. However, the decrease in the metabolism/uptake of anandamide by CB1-receptor antagonists[39] or facilitation of CB1-mediated pro-neurogenic responses[43] could also be involved.

Despite the results, certain limitations should be mentioned. This paper only reports the effects of CBD on subjective reports of anxiety, and not physiological effects, such as salivary cortisol levels or autonomic nervous system measures. Determining the effects of CBD on physiological responses to stress would have been a valuable addition to our findings. Since previous studies have shown that women present a greater arousing effect in SPST tests,[44] we decided to include only men in the present study and, thus, our findings cannot be generalized to women. Another relevant point is the relative similarity of CBD's effects at all doses, which suggests that a larger sample is needed to enhance the statistical data.

In conclusion, we observed again that single acute doses of CBD cause an inverted U-shaped dose-response pattern in human subjects submitted to an experimental model of anxiety. The narrow therapeutic window of CBD makes it difficult to use the drug in clinical settings before rigorous chronic dose-response clinical trials have established optimal doses. Therefore, determining adequate treatment ranges for clinical anxiety (and any other medical condition for which CBD can be considered a treatment option) remains a challenge. However, recent well-designed clinical trials with large samples involving the chronic administration of CBD have shown that it is possible to establish the therapeutic windows of this compound for different conditions.[7] Likewise, different preparations (e.g., inhaled, patches or i.v.) and combinations of cannabinoids can be produced in an attempt to optimize the therapeutic effects of CBD. Finally, further research is necessary to establish CBD's precise mechanisms of action in different disorders and to evaluate whether therapeutic drug monitoring would be helpful in optimizing CBD outcomes and safety.

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Disclosure

STI-Pharm (Brentwood, UK) kindly supplied CBD at no cost. AWZ, FSG, RM and JAC are co-inventors (Mechoulam R, JAC, Guimarães FS, AWZ, Breuer A) of the patent "Fluorinated CBD compounds, compositions and uses thereof" (Pub. no. WO/2014/108899, International Application no. PCT/IL2014/050023, Def. US no. Reg. 62193296; 29/07/2015; registered in Brazil at INPI on 19/08/2015 [BR1120150164927]). The Universidade de São Paulo (USP) has licensed the patent to Phytecs Pharm (USP Resolution no. 15.1.130002.1.1). USP has an agreement with Prati-Donaduzzi (Toledo, Brazil) to "develop a pharmaceutical product containing synthetic CBD and prove its safety and therapeutic efficacy in the treatment of epilepsy, schizophrenia, Parkinson's disease, and anxiety disorders." JAC and JECH have received travel support from and are medical advisors of BSPG-Pharm. The funding agency had no role in the study design or in the decision to submit the paper for publication. The other authors report no conflicts of interest.

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